

## Use of Electron Microscopy for Detecting the Environmental Contamination by Asbestos: Analysis of Sentinel Animal Lung Tissue

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The presence of asbestos in ophiolitic rocks determines the need to assess the risk of exposure for workers during the extraction and processing of greenstone and for the population residing in the municipalities near ophiolitic outcrops. Previous studies [1-3] showed that the amphibole-group asbestos minerals, tremolite, are the most common type of asbestos found in ophiolites of the Mount Reventino area in Calabria, Italy. An asbestiform, but unregulated, mineral belonging to serpentine mineral group was also detected in this area: fibrous antigorite contaminated from chrysotile [4].

In this work, the possible diffusion of fibers in the environment was assessed using sentinel animals as indicators of environmental pollution.

Ten lung samples of sheeps, goats and wild boars which come from Mount Reventino area and, as a control, two lung samples of unexposed sheeps were analysed. The preparation and analysis of lung tissue was performed using a protocol described in the report of National Health Institute [5]. Briefly, formalin-fixed lung tissue samples were digested using sodium hypochlorite. The suspension was filtered on a polycarbonate membrane. The counting, identification and size (width, length and aspect ratio) of all fibers detected on the membrane were performed by field emission scanning electron microscopy (FESEM) technique and energy dispersive spectrometer (EDS). Tremolite fibers were detected in all samples. Fibrous antigorite was also detected in some lungs. No corpuscles of asbestos were observed and no asbestos fibers were detected in the controls. The Figure 1 shows an example of tremolite fiber in the lung sample of a sheep, and the Figure 2 shows an antigorite fiber present in the lung of wild boar. The geometric mean of diameters, lengths and aspect ratio of fibers were 0.5  $\mu\text{m}$ , 7.0  $\mu\text{m}$  and 13.2 respectively. Many fibers were shorter than 5.0  $\mu\text{m}$  and thinner 0.1  $\mu\text{m}$ . Some lengths exceeded 30  $\mu\text{m}$ . The concentrations of fibers per gram of dry lung range from  $10^4$  to over  $10^6$ .

The presence of tremolite fibers in the lungs of the investigated animals confirms the spread of mineral fibers in the environment. The high resolution of FESEM allowed to detect fibers within to the nanometric range of 1-100 nm and to identify the mineral fibers by elemental analysis.

### References:

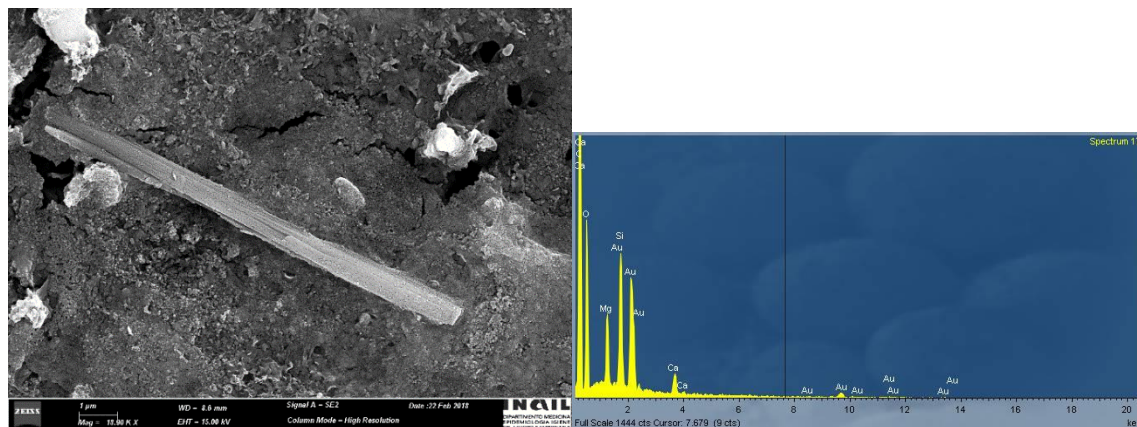
[1] A Bloise et al., *Environmental Earth Sciences* **71** (2014), p. 3773.

[2] A Bloise et al., *Italian Journal of Geosciences* **135** (2016), p. 268.

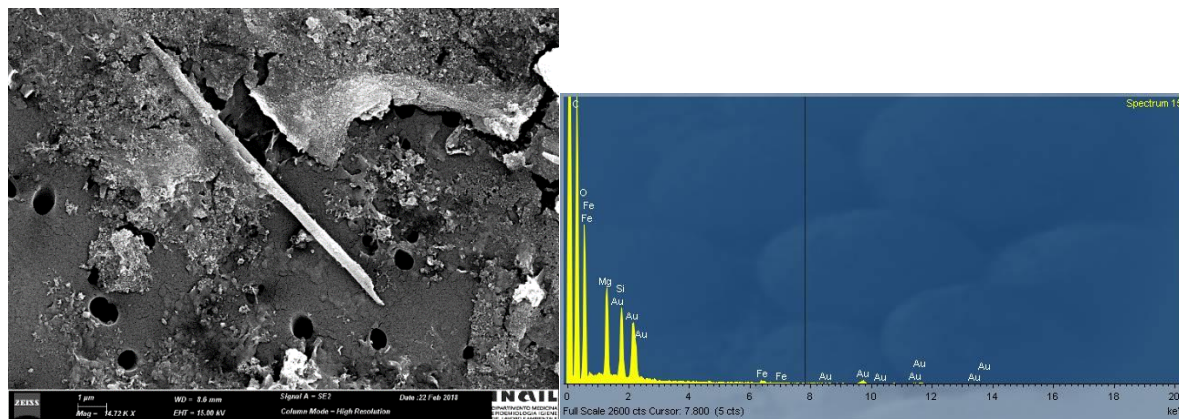
[3] A Campopiano et al., International Journal of Environmental Health Research, **28** (2018), p.134.

[4] A Campopiano et al., Journal of Mediterranean Earth Sciences **10** (2018), p. 17.

[5] Istituto Superiore Sanità, [http://old.iss.it/binary/publ/cont/17\\_12\\_web.pdf](http://old.iss.it/binary/publ/cont/17_12_web.pdf) (accessed February 18, 2019).



**Figure 1.** SEM image of tremolite fiber found in the lung sample of a sheet with EDS analysis.



**Figure 2.** SEM image of antigorite fiber found in the lung sample of a wild boar with EDS analysis.